Molecular Therapy
Oncology
Commentary

A novel oncolytic HSV co-expressing IL-12 and anti-PD-1 for glioblastoma

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Glioblastoma (GBM), the most common primary malignant brain tumor, is an invariably lethal cancer under the current standard of care.[1] Even systemic immune checkpoint blockade (ICB) anti-programmed death 1 (PD-1) immunotherapy, although approved in many other cancers, has failed in clinical trials for GBM.[1] The treatment failure is likely due to the immunosuppressive nature of the GBM microenvironment, referred to as "immunologically cold,"[1] which can be reversed by intratumoral administration of oncolytic herpes simplex virus (oHSV),[2,3] a promising anticancer approach approved in Japan for recurrent GBM.

Intralesional delivery of ICB and interleukin-12 (IL-12) can be effective against cancer preclinically, but both are toxic when given systemically.[4] Considering oHSV's capacity for foreign transgene expression, several oHSVs have been engineered for local co-expression of IL-12 and anti-PD-1. T3855, expressing IL-12 plus anti-PD-1, has shown promise in preclinical studies but has not been evaluated in GBM.[5] STI-1386 (Seprehvec), expressing IL-12, anti-PD-1 single chain fragment variant (scFv), and a transforming growth factor β receptor 2 decoy, is undergoing clinical trial, albeit not for GBM (ClinicalTrials.gov: NCT05361954). In this study, Wang et al. developed a similar oHSV co-expressing human IL-12 and anti-PD-1 antigen-binding fragment (C5252) and evaluated its oncolytic activity *in vitro* and safety and antitumor efficacy *in vivo* in GBM models.[6]

The C5252 virus efficiently produces IL-12 and anti-PD-1 upon infection of Vero cells. Compared to control R3616 (an oHSV with no transgene expression and lacking both copies of the *γ34.5* genes), C5252 (lacking both copies of the *γ34.5* genes and having a deletion of the internal repeat region, which could further attenuate the virus) shows reduced viral yields but higher cytotoxic activity in human GBM cells *in vitro*. It is not clear how lower replication correlates with increased cytotoxicity; one possible explanation could be that human IL-12, released from C5252 infection in the culture supernatant, may have induced cytotoxicity. C5252-induced oncolysis was associated with a significant increase in caspase-3/7 activity (vs. R3616 or wild-type HSV) via downregulation of ciliary neurotrophic factor receptor α(CNTFRα), a receptor for CNTF required for survival of neuronal cells.[6]

The safety of C5252 was confirmed in BALB/c nude mice. Mice injected intracranially with C5252 survived a significantly higher dose than wild-type HSV-1 (F strain) mice and exhibited no physical or behavioral abnormalities. Furthermore, C5252 did not establish latency, and its reactivation was reduced. C5252's median lethal dose ($>10^5$ plaque-forming units) and inability to establish latency suggest that it is safe for GBM treatment. These safety characteristics likely stem from the deletion of the neurovirulence gene *γ34.5* and the internal repeat.

In GBM xenograft models (both subcutaneous and orthotopic), intratumoral injections of C5252 demonstrated significantly better control of the tumor burden than R3616, which is somewhat surprising since both human IL-12 and anti-human PD-1 are not active in mice. This transgene inactivity and the lack of an intact immune system in xenograft models means that the immunotherapeutic potential of C5252 cannot be assessed preclinically. To address this, a murine version of C5252 (C8282) expressing murine IL-12 and anti-murine PD-1 was constructed and tested in immunocompetent syngeneic GBM models in mice.

In the orthotopic CT-2A-GFP-Luc model, intratumoral treatment with C8282 significantly extended median survival, with 25% long-term survivors, which is promising. However, the study lacked important control groups, such as unarmed oHSV, to distinguish the impact of transgene expression from oncolysis, and oHSVs expressing single transgenes, to define the roles of IL-12 or anti-PD-1 in treatment efficacy. In a somewhat artificial subcutaneous CT-2A-GFP-Luc model, C8282 treatment reduced the tumor burden significantly compared to its parental unarmed C1212, indicating a role of IL-12 and/or anti-PD-1, and increased intratumoral IFN-γ and tumor necrosis factor alpha. IL-12 and anti-PD-1 expression likely induced IFN-γ and recruited immune cells into the tumor, which may have contributed to antitumor efficacy (as depicted in Figure 1). However, the authors did not measure tumoral transgene expression or evaluate immune cell infiltration.

In conclusion, the authors have constructed new armed oHSVs (human C5252 and murine C8282) and demonstrated their safety and efficacy. Given the multifaceted immune mechanisms of IL-12 and anti-PD-1 in enhancing antitumor immunity (Figure 1), further research evaluating the effects of C8282 on the tumor immune microenvironment and the requirement for specific immune cells is necessary to understand the antitumor mechanisms of action of C5252/C8282.

AUTHOR CONTRIBUTIONS

K.A. prepared the figure. X.F. commented on this commentary. D.S. conceived and wrote the commentary.

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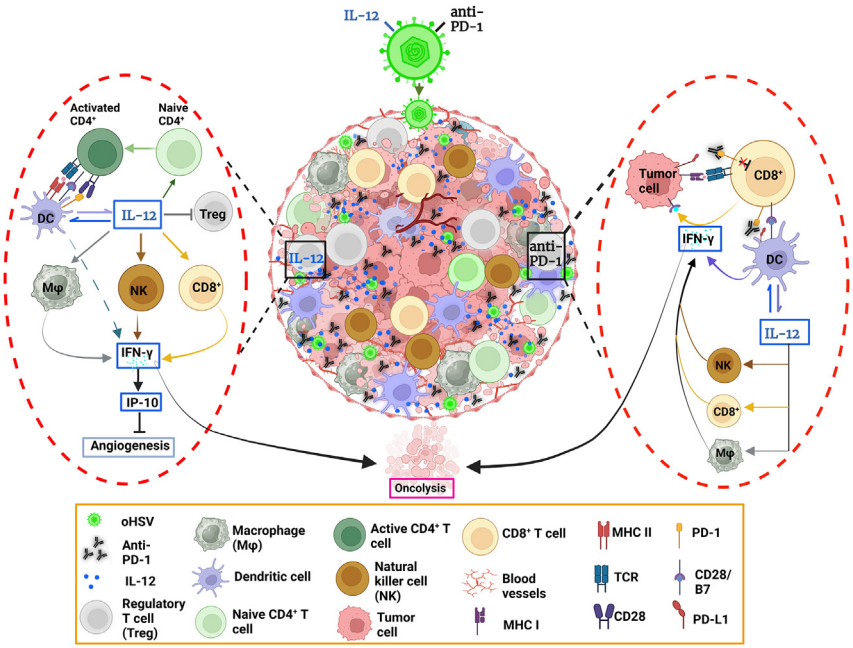

Figure 1. The likely antitumor immune mechanisms of action of intratumoral C8282 treatment

Center: following intratumoral injection, C8282 is anticipated to selectively replicate and kill cancer cells and release IL-12 and anti-PD-1 antibodies within the tumor microenvironment (TME). Oncolysis and viral release of IL-12/anti-PD-1 recruit various immune cells to the TME. Left: C8282-derived IL-12 enhances the activity of dendritic cells (DCs), natural killer (NK) cells, and $CD8^+$ T cells and polarizes naive $CD4^+$ T cells toward the activated Th1 phenotype and macrophages toward the M1 phenotype, which all contribute to the production of the interferon γ (IFN- γ) killer cytokine. IL-12 also inhibits the activity of immunosuppressive regulatory T cells. IL-12 stimulation and/or antigen presentation by $CD4^+$ T cells leads to the maturation of DCs, which, in turn, further produce IL-12 and IFN-γ (and other cytokines not listed here). The secreted IFN-γ destroys tumor vasculature via the IP-10 pathway and lyses tumor cells. This cyclic process continues until the tumor is eradicated or C5252 is cleared from the tumor by the host immune system. Right: programmed death 1 (PD-1) expression on T cells often helps tumor cells to evade immune surveillance by interacting with its ligands, PD-L1/-L2, resulting in T cell exhaustion. However, anti-PD-1 antibodies released from C8282 infection block PD-1:PD-L1/-L2 interaction, thus allowing T cells, such as $CD8^+$ T cells, to maintain effector function and sustain antitumor immunity, which includes, but is not limited to, activation of DCs, leading to the production of IFN-γ and IL-12. DC-derived IL-12 can further stimulate $CD8^+$ T cells, NK cells, and macrophages to produce more IFN-γ that exerts direct cytotoxic effects on cancer cells. This figure was generated with BioRender.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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